Elevated CO_2 increases shoot growth but not root growth and C:N:P stoichiometry of *Suaeda aralocaspica* plants

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Abstract: The purpose of the current study was to investigate the eco-physiological responses, in terms of growth and C:N:P stoichiometry of plants cultured from dimorphic seeds of a single-cell C_4 annual *Suaeda aralocaspica* (Bunge) Freitag and Schütze under elevated CO_2. A climatic chamber experiment was conducted to examine the effects of ambient (720 μg/L) and CO_2-enriched (1440 μg/L) treatments on these responses in *S. aralocaspica* at vegetative and reproductive stages in 2012. Result showed that elevated CO_2 significantly increased shoot dry weight, but decreased N:P ratio at both growth stages. Plants grown from dimorphic seeds did not exhibit significant differences in growth and C:N:P stoichiometric characteristics. The transition from vegetation to reproductive stage significantly increased shoot:root ratio, N and P contents, but decreased C:N, C:P and N:P ratios, and did not affect shoot dry weight. Moreover, our results indicate that the changes in N:P and C:N ratios between ambient and elevated CO_2 are mainly caused by the decrease of N content under elevated CO_2. These results provide an insight into nutritional metabolism of single-cell C_4 plants under climate change.

Keywords: biomass; CO_2 elevation; C:N:P stoichiometry; seed heteromorphism; *Suaeda aralocaspica*

1 Introduction

Since First Industrial Revolution, atmospheric CO_2 has steadily increased, and current concentration is above 720 μg/L and could reach 1440 μg/L by the year 2100 (IPCC, 2013; Betts et al., 2016). Such elevation in atmospheric CO_2 induces several alterations in plant metabolism. Growth and ecological responses of terrestrial plants to elevated CO_2 have been determined on three main types of photosynthesis: C_3, C_4 and crassulacean acid metabolism (CAM) types (Ceusters and Borland, 2011; Reich et al., 2018; Quirk et al., 2019). Responses of different plant functional types differ to elevated CO_2. In many C_3 and CAM plants, plant growth is stimulated by elevated CO_2 (Ceusters and Borland, 2011; Boretti and Florentine, 2019; Li et al., 2020). However, C_4 plants show little or no increase in productivity with elevated CO_2 (Boretti and

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Florentine, 2019). So far, there is no information available regarding responses of C_4 species without Kranz anatomy to atmospheric CO_2 elevation.

Some plants can produce heteromorphic seeds that are distinctly different in morphology, color, dormancy and germination traits (Imbert, 2002; Zhao et al., 2018). Plants cultured from heteromorphic seeds can also differ in their growth responses under different growth conductions, including biotic and abiotic factors (Redondo-Gómez et al., 2008; Jiang et al., 2020). However, the effects of elevated CO_2 on growth, ecological and physiochemical characteristics are still unknown for seed-heteromorphic plants.

Carbon (C), nitrogen (N) and phosphorus (P) contents and C:N:P ratio is associated with plant adaptation strategy to environmental changes (Du et al., 2019; Li et al., 2021). C, N and P provide the structural basis of plants and play essential role in photosynthesis and growth (Marschner, 2013). Though these elements are maintained at relative cellular concentration in plants, C:N:P stoichiometry of plants can be greatly affected under different environmental conditions (Deng et al., 2015). Numerous simulation experiments were carried out to study the response of elevated CO_2 on plant C:N:P stoichiometry (Pérez-Romero et al., 2018; Du et al., 2019; Uchytilová et al., 2019). However, the effects of elevated CO_2 remain inconsistent, for example, an average of 22% increase in the foliar C:N ratio in C_3 grasses and forbs was observed under high CO_2 concentration, but no significant effects were observed in C_4 grasses (Sardans et al., 2012).

Suaeda aralocaspica (Bunge) Freitag and Schütze is a habitant of the saline and semi-arid deserts in Central Asia (Commissione Redactorum Florae Xinjiangensis, 1994). This plant does not have dual-cell (Kranz) system, but has unusual mechanisms of C_4 photosynthesis by dimorphic chloroplasts in individual chlorenchyma cells via intracellular compartmentation (Voznesenskaya et al., 2001). In *S. aralocaspica*, photosynthetic chloroplasts are assembled at the proximal end in the flower and leaves, while other chloroplasts positioned and partitioned on the fringe of the cells at their distal end. In C_4 cycles, atmospheric CO_2 is fixed via phosphoenolpyruvate carboxylase (PEPC) at the distal end of the cell, which produces C_4 acids and donates CO_2 to rubisco in chloroplasts at the proximal end of the cell (Boyd et al., 2007).

We conducted a chamber experiment to examine the growth and C:N:P stoichiometry for plants grown from dimorphic seeds of this single-cell C_4 species to elevated CO_2. We hypothesized that plants from dimorphic seeds might have differential eco-physiological responses to CO_2 elevation at different growth stages.

2 Materials and methods

2.1 Seed collection and experimental design

Freshly matured *S. aralocaspica* fruits were harvested from the southern edge of the Junggar Basin in Xinjiang Uygur Autonomous Region, China, in October 2011. Fruits were naturally dried for 2 weeks at ambient room conditions. Dry fruits were rubbed by hand and both brown and black seeds were separated and stored in plastic bags at room temperature (20°C–25°C).

A pot experiment was conducted in two growth chambers (day/night air temperatures: 25°C/10°C for the first three months and then 30°C/15°C) at Fukang National Field Scientific Observation and Research Station for Desert Ecosystems, Chinese Academy of Sciences (44°17′N, 87°56′E; 460 m a.s.l.) in Xinjiang, China. The average concentration of CO_2 for growth chambers was either 720 or 1440 µg/L. The CO_2 levels were continuously controlled with an automatic CO_2 supply system (ESHENGTAIHE CTRL TECH, YB6, Beijing, China).

Twenty seeds of each brown and black seeds were sown into plastic pots (17 cm deep and 16 cm in diameter) filled with 2 L mix of quartz sand and vermiculite (3:1 v/v) at a soil depth of 2 mm. Two weeks after, one seedling was kept per pot, and seedlings with similar height and growth were kept for further experimentation. In each pot, 6 g of Osmocote 301 (Scotts, Marysville, OH, USA) was applied prior to sowing and 100 mL of Peters 1 nutrient solution was applied once a week. Salt stress was imposed at 4 weeks after sowing using a mixed salt $NaCl:Na_2SO_4:NaHCO_3$ (20:20:1). Each pot received 100 mL salt solution (1 g/ L) once a week

(Wang et al., 2012). Six plants grown from each seed type were harvested for the first time1157 under each CO_2 level on 15 July 2012. The second harvest took place on 15 September 2012.

2.2 Data collection

2.2.1 Plant biomass

After harvesting, shoot and root parts were separated and dried in the oven at 80°C for 48 h to reach a constant weight. Shoot and root biomasses were measured by an electronic analytical balance.

2.2.2 C, N and P content

Before chemical analysis, the dried shoot materials were ground in a ball mill, and then passed through a 0.25-mm sieve. The ball mill was cleaned completely after each sample milling. The shoot C content was determined using titration method after digested in $K_2Cr_2O_7$-H_2SO_4 (Bao, 2000). N content was determined using Kjeldahl method after digestion in H_2SO_4 -$HClO_4$ (Lin et al., 2011). P content was determined by colorimetric analysis (formation of chloro-phosphoric molybdate) after digestion in H_2SO_4-$HClO_4$ (Ren et al., 2018).

2.3 Statistical analyses

All data were expressed as mean±standard error. The data meet the assumptions of normality and homoscedasticity. Split-plot analysis of variance (ANOVA) was used to analyze the effects of CO_2 level and seed type on shoot and root dry weights, shoot C, N and P contents, and their ratios in *S. aralocaspica* plants, with CO_2 level (720 or 1440 μg/L) as main plot and seed type (brown and black seeds) as subplot at vegetative or reproductive stage. All data analyses were performed using SPSS v13.0 (SPSS Inc., Chicago, IL, USA).

3 Results

Elevation of CO_2 significantly increased shoot dry weight (SDW) both at vegetative and reproductive stages, but did not affect root dry weight (RDW) and shoot:root ratio. Plants grown from dimorphic seeds showed similar SDW, RDW and shoot:root ratio at the same growth stage under the same CO_2 condition (Fig. 1; Table S1). RDW decreased and shoot:root ratio increased during the transition from vegetative to reproductive stage (Fig. 1).

Elevation of CO_2 significantly decreased N content for plants grown from black seeds at vegetative stage, and did not affect other stoichiometric characteristics. Plants cultured from dimorphic seeds had the same C, N and P contents for both CO_2 treatments at the same growth stage (Fig. 2; Table S1). N and P contents in shoot significantly increased from vegetative stage to reproductive stage, however C content significantly decreased during this transition (Fig. 2). The range of C contents was between 309.4 and 330.4 mg/g. Plants from black seeds at elevated CO_2 showed the highest N (22.8 mg/g) and P content (3.7 mg/g) at reproductive stage (Fig. 2d and f).

Plants grown from dimorphic seeds had the same C:N, N:P or C:P ratio at same growth stage under the same CO_2 condition (Fig. 3). Compared with vegetative stage, plants at reproductive stage had lower C:N, C:P and N:P ratios (Table S1). Plants grown from black seeds at elevated CO_2 showed the lowest C:N ratio (14.3) and C:P (89.7) at reproductive stage (Fig. 3b and d). N:P ratio significantly decreased under elevated CO_2 in both plant types at vegetation stage (Fig. 3e).

4 Discussion

Although the growth and C:N:P stoichiometry of plants with different photosynthetic pathways subjected to elevated CO_2 have been studied extensively (Sardans et al., 2012; Deng et al., 2015; Du et al., 2019), our data are the first in which responses to CO_2 elevation have been documented for single-cell C_4 plants. In addition, our data indicate that elevated CO_2 significantly increases the shoot growth at both vegetative and reproductive stages, but not the root growth and C:N:P stoichiometry. The results also indicate that plants cultured from dimorphic seeds of *S. aralocaspica* have similar growth and C:N:P stoichiometry.

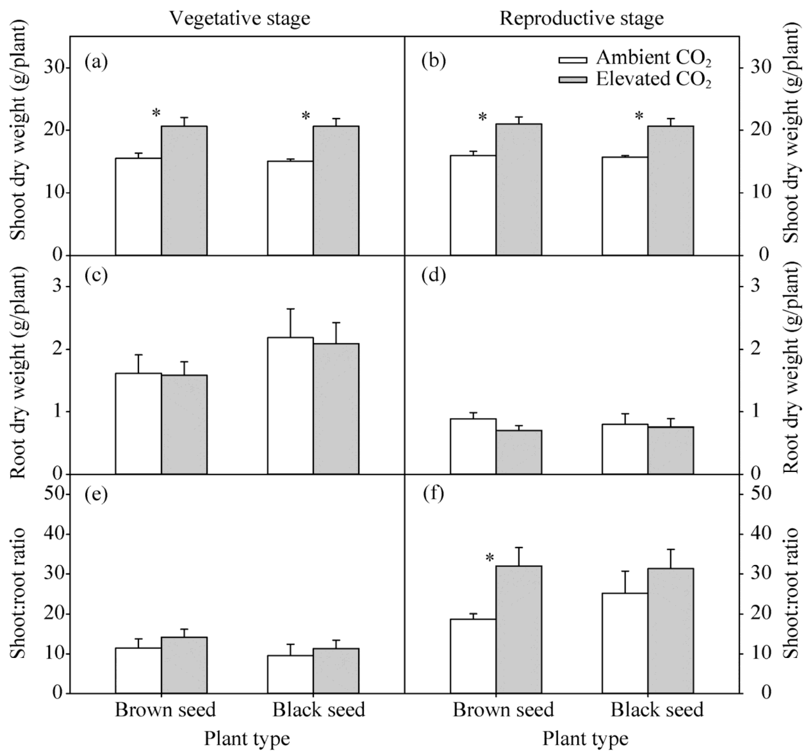

Fig. 1 Effects of elevated CO_2 on shoot dry weight (a and b), root dry weight (c and d), and shoot:root ratio (e and f) in plants grown from dimorphic seeds of *Suaeda aralocaspica* at vegetative and reproductive stages. * indicates significant difference between ambient and elevated CO_2 treatments at $P<0.05$ level.

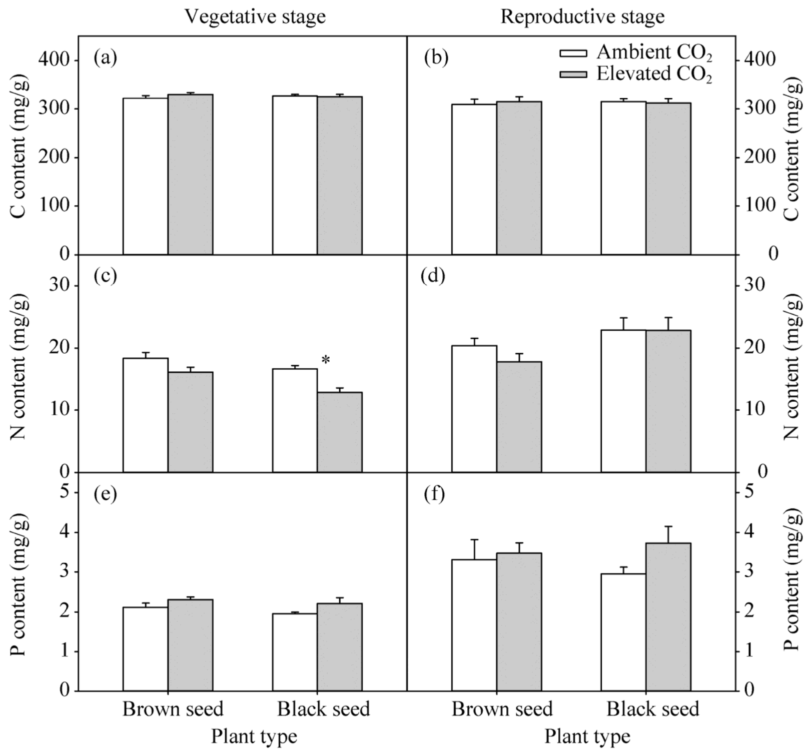

Fig. 2 Effects of elevated CO_2 on C content (a and b), N content (c and d), P content (e and f) in shoots of plants grown from dimorphic seeds of *Suaeda aralocaspica* at vegetative and reproductive stages. * indicates significant difference between ambient and elevated CO_2 treatments at $P<0.05$ level.

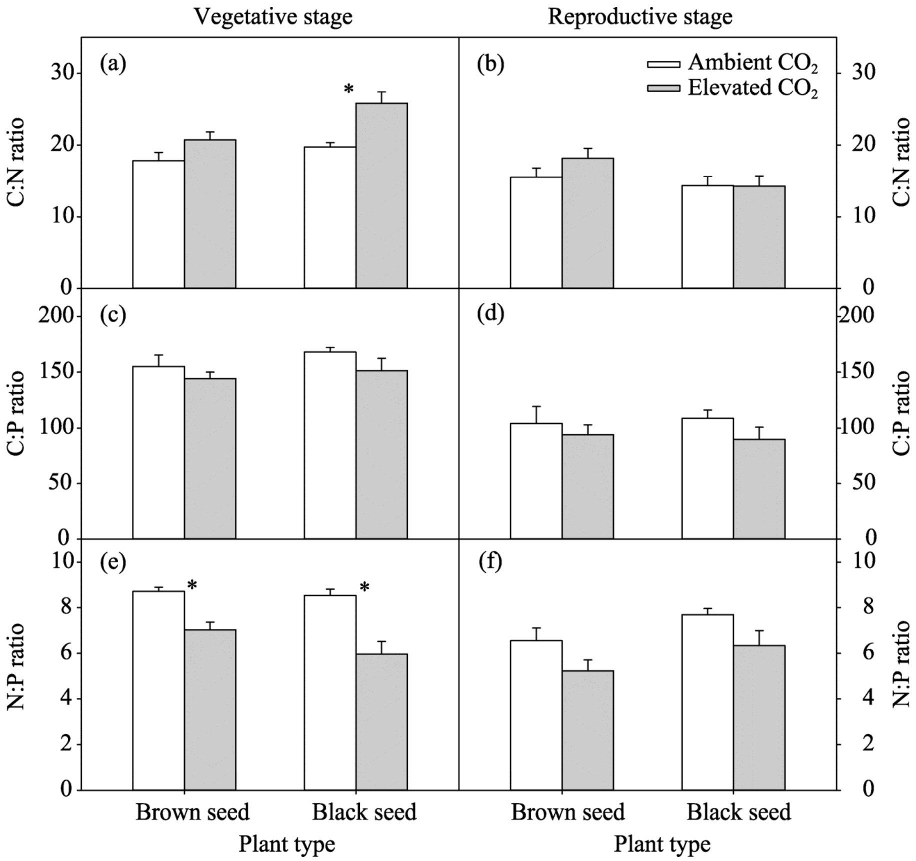

Fig. 3 Effects of elevated CO_2 on C:N ratio (a and b), C:P ratio (c and d), and N:P ratio (e and f) in shoots of plants grown from dimorphic seeds of *Suaeda aralocaspica* at vegetative and reproductive stages. * indicates significant difference between ambient and elevated CO_2 treatments at $P<0.05$ level.

Elevated CO_2 alters plant growth, contents of C, N and P, and C:N and N:P ratios (Li et al., 2019). Our results confirmed that elevated CO_2 significantly accelerated shoot growth and decreased N content of *S. aralocaspica*, which was consistent with previous studies. On average, N content in plant tissue decreases by about 14% under elevated CO_2 (Cotrufo et al., 1998). Elevated CO_2 has different effects on the N contents of different plant types and the reduction in N contents is smaller in C_4 plants than in C_3 plants. The changes in C:N and N:P ratios of *S. aralocaspica* between ambient and elevated CO_2 are mainly caused by the decrease of N content at elevated CO_2. The results coincide with previous results that C:N ratio increases under elevated CO_2 regimes (Sardans et al., 2012).

Differences exist not only in seed size and germination of heteromorphic seeds but also in plant growth characteristics and even maintain until the end of growing season (Imbert, 2002). Contrary to owning differences in seed dormancy, germination and plant reproduction (Wang et al., 2008, 2012), plants cultured from dimorphic seeds of *S. aralocaspica* did not significantly differ in their responses to CO_2 elevation. The differential response of heteromorphic seeds is effective in reducing the offspring survive risk in unpredictably fluctuating environments. For example, dimorphic seeds of *S. aralocaspica* show different salt tolerance (Wang et al., 2008). Because the steady increase of CO_2 concentrations is the same for plants grown from dimorphic seeds of *S. aralocaspica*, it is not surprising that both plant types show similar growth and C:N:P stoichiometric characteristics.

Our results showed that N and P contents increased with plant developmental stage. This could be explained by that plants at reproductive stage need a higher nutrient concentration to support

fruiting (Marschner, 2013). C content of *S. aralocaspica* was significantly lower than that (46.4% C) of global terrestrial plants (Elser et al., 2000). Decrease of C allocation at reproductive stage could be related to a high salt accumulation of this species.

5 Conclusions

Our results reinforce the conclusion that there is no difference between plants grown from heteromorphic seeds in the responsiveness of ecophysiology to steady environmental factors. Elevated CO_2 greatly decreased N content and then caused the change of N:P ratio of *S. aralocaspica*. Our results could enrich the plant response pattern of different types of photosynthesis to CO_2 elevation. If we want to accurately predict responses of various plants under future CO_2 concentration, further work is necessary to explore representative plants of special plant photosynthetic types, such as single-cell C_4 and C_3–C_4 intermediate plant species.

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Appendix

Table S1 Split plot variance analysis of the effects of elevated CO_2, plant type and their interactions on growth and C:N:P ratio

Growth stage	Index	CO_2		Plant type		CO_2×Plant type	
		F	*P*	*F*	*P*	*F*	*P*
Vegetative	Shoot	474.824	0.000*	0.031	0.864	0.034	0.858
	Root	0.046	0.839	4.020	0.073	0.016	0.902
	Shoot:root ratio	1.996	0.217	2.012	0.186	0.075	0.790
	C	0.897	0.387	0.003	0.956	0.947	0.353
	N	6.187	0.055	1.553	0.241	0.082	0.781
	P	4.076	0.099	1.679	0.224	0.002	0.963
	C:N ratio	6.031	0.058	2.021	0.186	0.132	0.724
	C:P ratio	3.937	0.104	1.398	0.264	0.111	0.746
	N:P ratio	28.070	0.003*	0.714	0.418	0.108	0.749
Reproductive	Shoot	51.556	0.001*	0.024	0.880	0.367	0.558
	Root	0.660	0.454	0.069	0.798	0.001	0.972
	Shoot:root ratio	4.184	0.096	0.483	0.503	0.737	0.411
	C	0.034	0.861	0.022	0.884	0.163	0.695
	N	1.652	0.255	0.026	0.876	0.791	0.395
	P	0.565	0.486	3.404	0.095	0.397	0.543
	C:N ratio	0.967	0.371	2.448	0.149	0.698	0.423
	C:P ratio	1.434	0.285	0.001	0.982	0.147	0.710
	N:P ratio	16.494	0.010*	4.097	0.070	0.001	0.971

Note: C, carbon; N, nitrogen; P, phosphrous. * indicates the significant difference at P<0.05 level.